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BRCA1 is a tumor-suppressor locus on chromosome 17q21. Familial inheritance of a defective copy places a lifetime risk of breast cancer at 80%. Most of these tumors arise before the age of 50. In addition, there is an elevated risk of ovarian and testicular tumors. The mechanism of transformation is not known. In an effort to better understand the actions of this gene, I have cloned the mouse *Brca1* gene. The present proposal aims to characterize the effect of over-expression and deletion of *Brca1* in mice, and by understanding the nature of the interactions between *Brca1* and other oncogenes. The completion of these aims will provide: (1) a mouse model of *Brca1* deficiency, (2) an enhanced understanding of *Brca1* function in the regulation of mammary epithelial cell growth and differentiation, and (3) reveal important interactions between *Brca1* and other potent transforming agents in the breast, in particular with c-*myc*, and loss of p53.

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Timothy F Lane 2/2/00

PI - Signature Date

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INTRODUCTION:

In our original application, we proposed to investigate the following specific aims:
1. Characterize of the effects of *Brca1* expression on the proliferation and differentiation of breast cancer cells of known genotype.
2. Establish mice that lack functional *Brca1* by targeted disruption.
3. Genetic complementation of *Brca1* deficient mice with strains that express oncogenes known to contribute to the development of breast cancer.

As reported in the previous two years we have made significant progress on all three aims. In particular, we have studied the effect of overexpressing BRCA1 in mouse mammary epithelial cells and have studied the subcellular localization of the murine Brca1 gene product (Aim 1). These experiments were completed and summarized in 1997 annual report.

As reported in 1998, we created a line of transgenic mice that overexpress human BRCA1 (MBR) (aim 1) and have created a second group of mice that overexpress an antisense construct of mouse Brca1(BAS) (aim 1). We have created a line of mice that carry an inactivated Brca1 locus (BrKO) (aim 2) and have crossed the BrKO mice with mice predisposed to cancer, including ($p53^{+/-}$, $p21^{+/-}$, and MMTV-myc) (Aim 3). In the course of these experiments, we experienced several technical difficulties which hindered our progress. In particular, BrKO mice do not develop cancer and mating with cancer prone $p53^{+/-}$, $p21^{+/-}$, or MMTV-myc stains did not appear to accelerate or contribute to tumor progression of these strains.

In this final report we will summarize our results with BAS (MMTV-Brca1 antisense) mice which were substituted for BrKO mice in Aim 3. We have crossed the BAS mice into $p53^{+/-}$ and MMTV-c-neu backgrounds. As described below, we find that BAS mice are tumor prone and that tumor incidence is accelerated in p53null backgrounds. Interestingly, tumor progression was unchanged, or even delayed in BASx MMTV-c-neu mice. These results are discussed in the context of information on the incidence of p53 mutations and erbB2 amplifications observed in human BRCA1 associated breast cancers.

BODY:

Analysis of tumor formation in Brca1 knock-out (BrKO) mice:

As described in the 1997 and 1998 progress report, BrKO mice were established on the 129^{SvEv} genetic background and have been monitored since 1996. In all respects, the phenotype of these mice appears similar to that described by other groups who have knocked out the Brca1 gene (6, 8, 11, 12). Since that time we have mated the line with FVB-N and backcrossed into FVB to create a syngeneic strain on the FVB genetic background. Homozygous $BrKO^{FVB}$ mice ($BrKO^{FVB}/BrKO^{FVB}$) display embryonic lethality at day 8-9 of development and thus are not useful for analysis of tumor progression. We also fail to identify tumors in heterozygous animals ($BrKO^{FVB+/-}$), a result consistent with other groups. To date, we have analyzed over 400 mice that reached 12 mo of age or greater.

Generation of Transgenic mice expressing MMTV-Brca1 Antisense (TgN-MMTV-BAS) and MMTV-BRCA1:

A) To overcome the long latency in tumor progression in $BrKO^{+/-}$, we generated several lines of transgenic mice expression a Brca1 antisense (TgN-MMTV-BAS) construct targeted to the mammary gland (Table #1). The justification and strategy for creating these mice was described in the 1997 progress report.

B) We also tried to generate several dominant overexpresser lines using human BRCA1 cDNA's linked to various ubiquitous (CMV, b-actin) or tissue specific (MMTV) promoters but never recovered successfully integrated founder animals. One founder that carried an MMTV-BRCA1 cDNA was recovered. Later analysis of tissues from offspring of this animal showed no expression and the line was discontinued. We concluded that inappropriate expression of BRCA1 was detrimental to murine development and have initiated a second line of research to evaluate BRCA1 promoter elements that can be used to direct the expression of transgenes.

The new lines that were created are described in Table 1.

Table 1 Transgenic mice (new lines)

Construct/transgene	Name	# of lines[1]	tumor formation	Transgene expression
MMTV-Brca1 antisense	BAS	8	yes (3/8 lines)	yes (3/3 tested)
MMTV-BRCA1	MBR	1	no	no
beta-actin-BRCA1		0	N.A.[4]	N.A
CMV-BRCA1		0	N.A.	N.A

[1]Number of lines represents the number of founders that transmitted transgenic DNA to offspring.
[4]Not applicable, this construct appears to result in embryonic lethality.

TgN-MMTV-BAS mice (BRCA1 antisense) have been in the lab for 3.5 years. Upon dexamethasone treatment, 3 week old BAS females show evidence of mammary hyperplasia with increased numbers of branch points in the mammary tree (see Figures 1 and 2 from the last annual report). Non-treated (dexamethasone free) females develop mammary adenocarcinomas with long latency (6-12 months (Figure 2). The incidence is low (4/20 mice > 8mo of age). We have now mated these mice into a p53 null background. Bigenic BAS/p53 colony develop mammary adenocarcinoms much more rapidly (3-4 months) and the tumors have a characteristic increase in vascularity. The incidence of mammary tumors is significantly increased over p53 alone indicating that the BAS transgene collaborates with loss of p53 in tumor formation. The strategy of targeting Brca1 antisense (BAS) appears to be a more effective means of eliminating Brca1 expression than waiting for allelic loss at the endogenous Brca1 locus in BrKO mice. It therefore is a very promising alternative for studying BRCA1 induced tumors.

We have also generated a number of BASxMMTV-c-neu mice. Interestingly, in over 20 bigenic animals observed to date, tumors develop with longer latency than do MMTV-c-neu monogenic mice. Also, do MMTV-c-neu monogenic mice usually develop multiple tumor foci by 4-6 months whereas the bigenic mice usually only present with a single tumor mass. We are pursuing this result as it might help explain the lack of erbB2 amplifications reported in human BRCA1 mediated breast cancers.

We have established cell lines from the BAS mice in the hopes of demonstrating reduced Brca1 protein expression. Unfortunatley, commercially available anti-mouse Brca1 protein antibodies (Santa Cruz) fail to work well in our hands. We have developed an very sensitive RNase protection assay which we hope will allow us to prove the mechanism.

Conclusions:

At the end of year 3, we have made progress on all 3 Aims. We have shown that mouse Brca1 is a nuclear protein that blocks cell proliferation when overexpressed. We have also shown that Brca1 is an essential gene. Loss of the gene in BrKO mice results in early

embryonic lethality. Heterozygous BrKO animals are healthy and do not appear to show increased susceptibility to breast cancers, or to any other disease states. While the lack of disease in BrKO mice has been disappointing, the Brca1 antisense (BAS) approach appears to be working. Specifically, we appear to be able to reduce Brca1 protein levels to the point were we can observe increased proliferation (hyperplasias) without inducing cellular lethality. We will continue characterizing these mice. To date 3 out of 8 BAS lines have developed at least one mammary tumor and we are particularly focused on line G which appears particularly cancer prone.

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